

### REMARKS

Upon entry of the present amendments, claims 1, 2, 5-8 and 11 are pending in the present application. Claim 4 has been canceled without prejudice. Claims 1, 2 and 5 have been amended. Support for the amendment to claim 1 can be found, for example, in claim 2 and at page 235, lines 15-16 of the substitute specification. No new matter has been added by the present amendments.

#### **Rejection under 35 U.S.C. §112, second paragraph**

The Examiner has rejected claims 1, 2, 4-8 and 11, on page 3, paragraph 1 of the Office Action, for being indefinite for reciting “or a complement thereof”. The Examiner notes inconsistencies of use of the comma in claims and of “the” versus “a”. Applicants have canceled claim 4, thus the rejection is moot as it regards claim 4. Applicants have amended claim 1 to place a comma before “or a complement thereof,” to make claim 1 consistent with the language of claim 5. Applicants have amended claim 2 to delete the language regarding a complement thereof. Applicants have further amended claim 5 to replace “a” with “the” before, “complement thereof.”

Moreover, Applicants agree with the interpretation of the claim language the Examiner asserts in the Office Action. Applicants define “a complement thereof” as it appears in the claims to encompass complementary sequences wherein the length may be different from the reference sequence and in which mismatches from the reference sequence could be incorporated as well. Applicants define “the complement thereof” as it appears in the claims to encompass complementary sequences of exactly the same length as the reference molecule.

Despite pointing out these inconsistencies, the Applicants do not see a specific rejection of these claims for indefiniteness, but merely a notice of the comma inconsistency, which Applicants have amended, and a definition of the phrases “a/the complement thereof”.

Applicants assert that claims 1, 2, 5-8 and 11 are definite. Further, Applicants have added the limitation, "wherein the complement is at least 10 nucleotides in length," and have further added specific stringency conditions for the complement to claim 1. Applicants submit that claims 1, 2, 5-8 and 11 are definite in light of these amendments. Absent any specific rejection by the Examiner, Applicants request that this rejection be withdrawn.

### **Rejection under 35 U.S.C. § 101**

The Examiner has rejected claims 1, 2, 4-8 and 11 on page 3, paragraph 3 of the Office Action for lack of utility. The Examiner asserted in the Office Action that there is no single substantial utility that is commonly shared among the members of the family of ORX proteins. Applicants traverse.

Substantial utility is defined in MPEP Section 2107.01.

#### **Substantial Utility**

A "substantial utility" defines a "real world" use. Utilities that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use are not substantial utilities. For example, both a therapeutic method of treating a known or newly discovered disease and an assay method for identifying compounds that themselves have a "substantial utility" define a "real world" context of use. An assay that measures the presence of a material which has a stated correlation to a predisposition to the onset of a particular disease condition would also define a "real world" context of use in identifying potential candidates for preventive measures or further monitoring.

As explained on page 118, line 53 on the substitute specification, SEQ ID NO:224 encodes a member of the olfactory receptor family of proteins. These proteins are chemoreceptors which enable mammals to smell (*see* page 1 of the substitute specification). This is a single substantial utility that is shared by the members of the olfactory receptor family. The relevant information arising from the nucleic acid sequence of the specific member of the olfactory receptor family claimed here (SEQ ID NO:224), is that it is a member of the olfactory receptor family of proteins.

Being a member of the olfactory receptor family of proteins, *i.e.* being a chemoreceptor that enables mammalian olfaction has inherent substantial utilities specific to the family members. For example, the protein encoded by SEQ ID NO:224 could be used to test whether potentially toxic compounds were capable of being detected by mammalian olfaction, by assaying binding of compounds to the protein (*see* page 273, lines 10-31 of the substitute specification). Further, it has been shown that the number of pseudogenes within the family of olfactory receptors in a specific mammalian species correlates with the sensitivity of the sense of smell in the species (*see* Roquier *et al.* PNAS USA 97(6) 2870-4 (March 14, 2000) attached as Exhibit A and page 2, lines 4-6 of the substitute specification). Thus, SEQ ID NO:224 could be used to ascertain the olfactory ability of mammalian species, depending on whether genes corresponding with SEQ ID NO:224 in other species were pseudogenes or not. All members of the olfactory receptor gene family share these substantial utilities. Thus, SEQ ID NO:224, as a member of this family, has a substantial utility.

SEQ ID NO:224 may be termed a “research tool” in light of the above mentioned utilities, but does not require further research to ascertain its utility. A substantial utility does not include utilities that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use are not substantial utilities (*see* MPEP 2107.01). However, substantial utilities do encompass research tools. MPEP 2107.01 describes research tools as follows.

Some confusion can result when one attempts to label certain types of inventions as not being capable of having a specific and substantial utility based on the setting in which the invention is to be used. One example is inventions to be used in a research or laboratory setting. Many research tools such as gas chromatographs, screening assays, and nucleotide sequencing techniques have a clear, specific and unquestionable utility (e.g., they are useful in analyzing compounds). An assessment that focuses on whether an invention is useful only in a research setting thus does not address whether the

invention is in fact "useful" in a patent sense. Instead, Office personnel must distinguish between inventions that have a specifically identified substantial utility and inventions whose asserted utility requires further research to identify or reasonably confirm. (Emphasis added).

As described above, SEQ ID NO:224 is useful for analyzing compounds and organisms and may thus be termed a "research tool" as defined in MPEP 2107.01. These above described utilities have a specifically identified substantial utility and do not require further research to identify or reasonably confirm their utility, *i.e.* SEQ ID NO:224 is a member of the olfactory receptor family which mediates olfactory sensation in mammals via binding to various chemicals and so family members can be used to identify these chemicals, and also rate olfaction in mammals which possess or lack family members.

Thus, Applicants submit that, one substantial, specific and real world utility of SEQ ID NO:224 is as a research tool to analyze compounds for their ability to be sensed by mammals. Another substantial, specific and real world utility for SEQ ID NO:224 is as a research tool used to detect the olfactory abilities of species. Since SEQ ID NO:224 has at least one substantial, specific and real world utility, Applicants request that this rejection be withdrawn.

**Rejections under 35 U.S.C. §112, first paragraph**

The Examiner has rejected claims 1, 2, 4-8 and 11 on page 4, paragraph 3 of the Office Action for lack of enablement. Applicants have canceled claim 4, thus this rejection is moot as it regards claim 4. The Examiner, first asserts that claims 1, 2, 4-8 and 11 lack enablement because they lack utility, so that one of ordinary skill in the art would not know how to operate the invention as intended. The utility of the invention of claims 1, 2, 5-8 and 11 has been shown above. Therefore, one of ordinary skill in the art would know how to operate the invention of claims 1, 2, 5-8 and 11 as intended.

Further, the Examiner has rejected claims 1, 2, 4-8 and 11 for lack of enablement in light of the meaning of the phrase “a complement thereof”. The Examiner has asserted, and Applicants agree, that the phrase, “a complement thereof” recited in the claims encompasses complementary sequences wherein the length may be different from the reference sequence and in which mismatches from the reference sequence could be incorporated as well. Applicants assert that one of ordinary skill in the art would be able to readily make and use these variants.

Claim 1 has been amended so that the complement must be at least 6 nucleotides in length and that it must hybridize to a nucleic acid sequence comprising SEQ ID NO:224 under stringent hybridization conditions of 6X SSC at 65 °C. Claim 1 and its dependent claims 2, 6-8 and 11 are enabled because one of ordinary skill in the art would be able to make and use these complements without undue experimentation.

Testing whether a nucleic acid sequence stringently bound to a nucleic acid sequence comprising SEQ ID NO:224 would not require undue experimentation. To ascertain whether experimentation is undue, the *Wands* factors are used.

These factors include, but are not limited to:

- (A) The breadth of the claims;
  - (B) The nature of the invention;
  - (C) The state of the prior art;
  - (D) The level of one of ordinary skill;
  - (E) The level of predictability in the art;
  - (F) The amount of direction provided by the inventor;
  - (G) The existence of working examples; and
- In re Wands*, 858 F.2d 731, 737, (Fed. Cir. 1988).

Relevant factors D, E and F as applied, here, show that experimentation is not undue. The hybridization of nucleic acid sequences is well within the level of knowledge of one of ordinary skill in the art and is predictable. The conditions of stringency are specific, thus the inventor gives sufficient direction. Based on homology of a given nucleic acid sequence to SEQ

ID NO:224 one of ordinary skill in the art could predict sequences likely to hybridize to it, and could easily use the stringency conditions stipulated in claim 1 to see if the given nucleic acid sequence was a complement of SEQ ID NO:224. Therefore, Applicants submit that there is no undue experimentation required for one of ordinary skill in the art to practice the invention, and thus the complement of claims 1, 2, 6-8 and 11 is enabled.

In light of the above, claims 1, 2, 6-8 and 11 are enabled. Claim 4 has been canceled, thus this rejection is moot as it regards claim 4. Applicants have amended claim 5 to replace “a” with “the” before “complement thereof,” so the phrase, “a complement thereof” is not at issue in claim 5. Thus Applicants submit that claims 1, 2, 5-8 and 11 are enabled and, therefore, request that this rejection be withdrawn.

**Rejection under 35 U.S.C. §102(b)**

The Examiner rejected claims 1, 2, 4, and 5 on page 5, paragraph 1 of the Office Action for being anticipated by The SIGMA PRODUCT CATALOGUE, 1993, page 743 for reciting the sequence GGG, which would complement the sequence of claims 1, 2, 4 and 5. Applicants have canceled claim 4, so this rejection is moot as it regards claim 4.

Applicants have amended claim 1 from which claim 2 depends, to stipulate that the complement must be 10 nucleotides in length and that it must stringently bind to a nucleic acid sequence comprising SEQ ID NO:224 under conditions of 6X SSC at 65 °C. The complement to GGG disclosed in The SIGMA PRODUCT CATALOGUE does not read on claim 1 as amended, and thus also does not read on dependent claim 2.

Applicants have further amended claim 5 to replace the “a” before, “complement thereof,” with “the”. As asserted by the Examiner, and agreed with by the Applicants on page 3, paragraph 2 of the Office Action, “the complement thereof” as it appears in the claims

encompasses complementary sequences of exactly the same length as the reference molecule. Claim 5 stipulates that the reference molecule must be at least 6 nucleotides long. Therefore, the complement stipulated in claim 5 must also be 6 nucleotides long. The complement to GGG disclosed in The SIGMA PRODUCT CATALOGUE does not read on claim 5 as amended.

Applicants have above asserted that The SIGMA PRODUCT CATALOGUE does not read on claims 1, 2 or 5 and thus cannot anticipate them. Thus, Applicants request that this rejection be withdrawn.

### CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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# The olfactory receptor gene repertoire in primates and mouse: Evidence for reduction of the functional fraction in primates

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Olfactory receptors (ORs) located in the cell membrane of olfactory sensory neurons of the nasal epithelium are responsible for odor detection by binding specific odorant ligands. Primates are thought to have a reduced sense of smell (microsmatic) with respect to other mammals such as dogs or rodents. We have previously demonstrated that over 70% of the human OR genes have become nonfunctional pseudogenes, leading us to hypothesize that the reduced sense of smell could correlate with the loss of functional genes. To extend these results, we sampled the OR gene repertoire of 10 primate species, from prosimian lemur to human, in addition to mouse. About 221 previously unidentified primate sequences and 33 mouse sequences were analyzed. These sequences encode ORs distributed in seven families and 56 subfamilies. Analysis showed a high fraction ( $\approx 50\%$  on average) of pseudogenes in hominoids. In contrast, only  $\approx 27\%$  of OR genes are pseudogenes in Old World monkeys, and New World monkeys are almost free of pseudogenes. The prosimian branch seems to have evolved differently from the other primates and has  $\approx 37\%$  pseudogene content. No pseudogenes were found in mouse. With the exception of New World monkeys, we demonstrate that primates have a high fraction of OR pseudogenes compared with mouse. We hypothesize that under relaxed selective constraints, primates would have progressively accumulated pseudogenes with the highest level seen in hominoids. The fraction of pseudogenes in the OR gene repertoire could parallel the evolution of the olfactory sensory function.

olfaction | pseudogenes | evolution

Mammals are able to discriminate between thousands of odor molecules. This capacity relies on a multigene family encoding 500–1,000 olfactory receptors (ORs; ref. 1). These receptors are expressed mainly in the olfactory epithelium and have been found in a number of species including mammals (1–5), birds (6, 7), amphibians (8), and fish (9). All these receptors belong to the G protein-coupled receptor superfamily and share features of sequence and structure, such as seven hydrophobic transmembrane domains.

The sense of smell plays an important role in mammalian social behavior, location of food, and detection of predators. However, mammals vary in their olfactory ability (10, 11). The sense of smell in primates is greatly reduced (microsmatic) with respect to other mammals such as dogs (12) or rodents (10, 11). Various explanations for the differences in olfactory performance have been hypothesized. Differences in the anatomical structures (e.g., size and location) devoted to olfaction could partly explain these differences. For example, dogs, which have an olfactory sensitivity up to 100 times greater than humans, have on average  $\approx 100\text{ cm}^2$  of olfactory epithelium, whereas humans have only  $10\text{ cm}^2$  (see ref. 4 and references therein). Variations in the size and diversity of the expressed OR gene family could also account for these differences. We recently demonstrated that the human OR gene repertoire is distributed in over 25 chromosomal sites, and over 70% of these OR genes

are pseudogenes, i.e., the sequences have accumulated deleterious mutations such as in-frame stop codons and/or indel frameshifts (3). This finding led us to hypothesize that the reduction of the sense of smell observed in primates could parallel the reduction of the number of functional OR genes.

To test this hypothesis, we wished to characterize the evolution of the OR gene family in other primates. We performed a random survey of OR genes from primate hominoids to prosimians. In parallel, we constructed a mouse OR-enriched library from genomic DNA to sequence a number of OR. The comparison of the OR gene repertoire from macrosmatic mouse and primates provides insight into the evolution of this multigene family and could reflect the evolution of a sensory function in mammals in response to selective constraints.

## Materials and Methods

### Cloning and Analysis of OR-Like Sequences in Primates and Mouse.

The isolation of OR-related sequences has been described elsewhere (3, 13). Briefly, 100 ng of genomic DNA from each species was subjected to PCR by using consensus OR primers OR5B-OR3B [OR5B (TM2), 5'-CCCATGTA(T/C)TT-(G/C/T)TT(C/T)CTC(A/G/T)(G/C)(C/T)AA(C/T)-(T/C)T(G/A)TC-3'; PMY(F/L)FL(S/A/T/G/C)NLS; OR3B (TM7), 5'-AG(A/G)C(A/T)(A/G)TAIATGAAIGG-(A/G)TTCAICAT-3'; M(L/F/V/I)NPF(I/M)Y(S/C)L; ref. 14]. A second pair of consensus primers, OR3.1-OR7.1 [OR3.1 (TM3), 5'-GCIATGGCITA(C/T)GA(C/T)(A/C)GITA-3'; AMAYD(S/R)Y; OR7.1 (TM7), 5'-A(A/G)I(G/C)(A/T)(A/G)TA(A/G/T)AT(A/G)AAIGG(A/G)TT-3'; NPFII-(S/R/T/C/W)(L/F); refs. 15 and 16], was also used to amplify primate OR sequences. PCR products were subcloned in the TA vector (Invitrogen), and recombinant clones were identified by PCR. Sequencing of the OR sequences was performed, and sequences were assembled and analyzed as detailed elsewhere (3). The following species were studied: human (*Homo sapiens*, HSA), chimpanzee (*Pan troglodytes*, PTR), gorilla (*Gorilla gorilla*, GGO), orangutan (*Pongo pygmaeus*, PPY), gibbon (*Hylobates lar*, HLA), macaque (*Macaca sylvanus*, MSY), baboon (*Papio papio*, PPA), marmoset (*Callithrix jacchus*, CJA), squirrel monkey (*Saimiri sciureus*, SSC), and *Saimiri boliviensis*, SBO),

Abbreviations: OR, olfactory receptors; HSA, *Homo sapiens*; PTR, *Pan troglodytes*; GGO, *Gorilla gorilla*; PPY, *Pongo pygmaeus*; HLA, *Hylobates lar*; MSY, *Macaca sylvanus*; PPA, *Papio papio*; CJA, *Callithrix jacchus*; SSC, *Saimiri sciureus*; SBO, *Saimiri boliviensis*; EFU, *Eulemur fulvus*; ERU, *Eulemur rubriventer*; MMU, *Mus musculus domesticus*; DRE, *Danio rerio*; ASI, amino acid identity.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF022649, AF073959–AF073989, AF127814–AF127907, and AF179716–AF179846).

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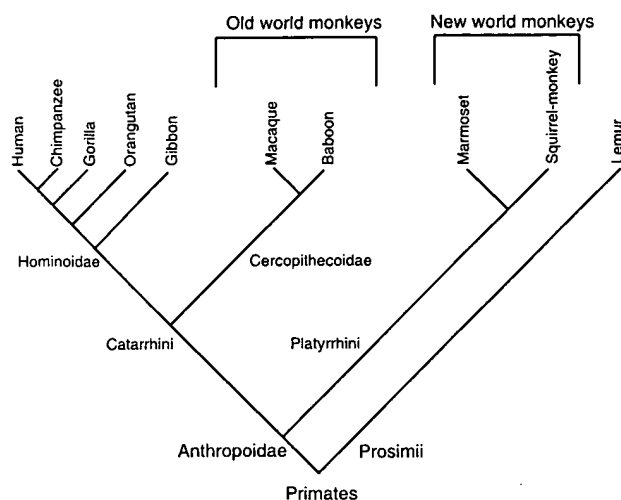


Fig. 1. Schematic phylogeny tree of the primate species used in the present study (adapted from ref. 32).

lemur (*Eulemur fulvus*, EFU, and *Eulemur rubriventer*, ERU), and mouse (*Mus musculus domesticus*, MMU). In addition, a few zebrafish (*Danio rerio*, DRE) sequences were characterized with primers OR3.1-OR7.1.

Pairwise sequence comparisons and multiple alignments were performed with GAP and PILEUP from the GCG package (Wisconsin Package, version 8).

**Construction and Screening of an OR-Specific Mouse Sublibrary.** Mouse OR clones obtained by PCR as described above were gridded in 96-well microtiter dishes (1,536 clones in eight plates). For hybridization screening, the clones were robot-spotted in duplicate on high-density filters as described elsewhere (17). Approximately 90% of the clones were identified as OR genes. This library was screened to identify clones hybridizing to human OR pseudogene sequences. Human plasmid DNA probes were radiolabeled to a specific activity of  $10^8$ – $10^9$  cpm/ $\mu$ g by random hexamer priming (18) by using [ $\alpha$ - $^{32}$ P]dCTP (Amersham Pharmacia). Filter hybridizations were carried out under standard hybridization conditions (19) and exposed to Kodak x-ray film at  $-80^\circ\text{C}$ . Three human OR probes were used: OR1-72, OR912-47, and OR15-71 (GenBank accession nos. U86218, U86230, and U86296, respectively).

## Results

**Isolation and Analysis of Primate OR Sequences.** To sample the OR genes in primate species, we randomly sequenced OR genes from anthropoids and prosimians (Fig. 1). OR genes were obtained by PCR on genomic DNA from the different species by using consensus OR primer pairs OR5B-OR3B and OR3.1-OR7.1 chosen, respectively, in the transmembrane domains TM2 and TM7 as well as TM3 and TM7. Except human, 18–35 individual OR clones were sequenced per taxon. A total of 221 OR sequences, representing 10 species, was analyzed. These sequences are distributed in different groups whose percentages of nucleotide sequence identity range from  $\approx 35$  to  $>99\%$  (not shown). The corresponding amino acid sequences were compared with a variety of OR sequences from the public databases and previous studies (3). All sequences have the characteristic features of ORs, with a heptahelical structure and conserved motifs as defined (1, 3, 14). The use of two pairs of consensus primers made our sampling representative of the OR gene repertoire. Primate sequences are distributed in seven families [sequences that share  $>40\%$  amino acid identity (ASI) define a

family] and 56 subfamilies (ASI  $>60\%$ ), with group 1-II of family 1 representing the zone of overlap of sequences derived by using the two primer pairs (Fig. 2). Nonhuman primate OR genes are represented in six families and about 45 subfamilies. Numerous sequences are grouped in family 1 ( $\approx 66\%$ ) comprising subfamily 1A, the largest subfamily (57 of 221 or 26%). Subfamily 1B is almost devoid of coding human OR sequences (Fig. 2). Subfamily 1A contains only human pseudogenes originating from chromosomes 14 and 19 (not shown), whereas subfamily 1B contains human pseudogenes lying on various chromosomes (not shown; ref. 3). As we found previously for human (3), the amino acid sequences deduced from the nonhuman primate sequences identified many pseudogenes (Fig. 2 and Table 1). Table 1 provides information about the evolution of the pseudogene fraction along with the evolution of primates. Hominoids present the highest fraction of pseudogenes (39 to  $>70\%$ ; average  $\approx 50\%$ ). Old World monkeys (macaque and baboon) have a lower pseudogene fraction (20 to 35%; average 27%), whereas even fewer pseudogenes were found among the sequences derived from New World monkeys. Only one pseudogene (SBO64) was identified among the 49 sequences obtained from marmoset and two species of squirrel monkey. In contrast, 37% of the prosimian lemur OR sequences were pseudogenes.

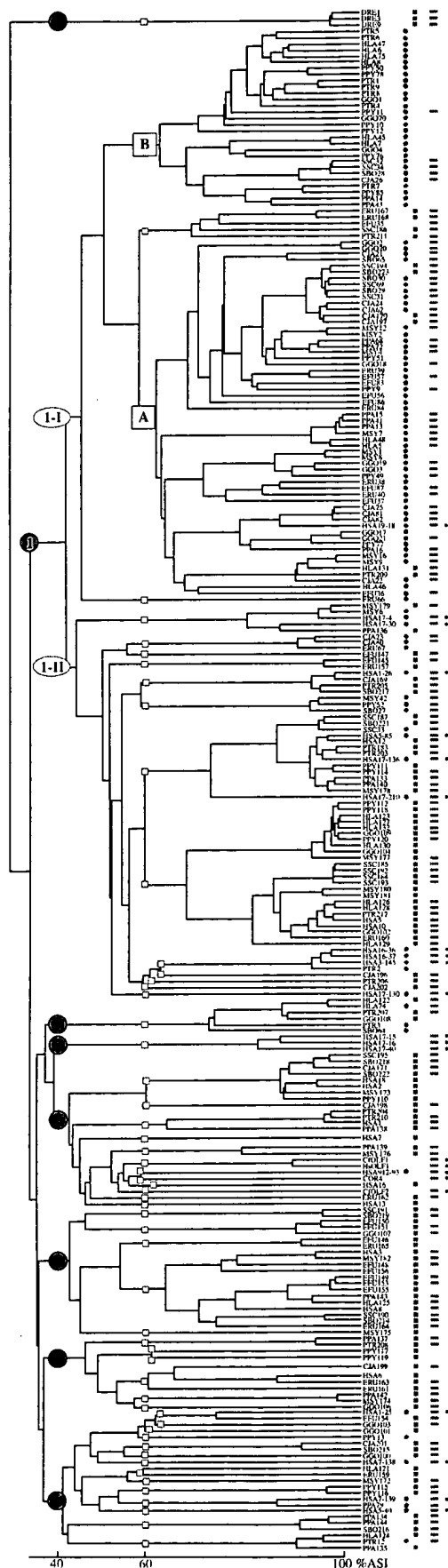
**Sequence Analysis of Mouse OR Sequences.** To test whether mammals thought to be microsmatic or macrosmatic differ in the fraction of pseudogenes in their OR repertoire, we surveyed OR sequences in the mouse genome. We constructed a mouse sublibrary enriched for OR-related sequences amplified by PCR from the mouse genome (see *Materials and Methods*).

Randomly selected mouse OR clones ( $n = 19$ ) were sequenced. All 19 have an uninterrupted ORF and are potentially functional. These sequences group primarily in family 1 (not shown) and vary from  $\approx 52$  to  $>99\%$  nucleotide sequence identity (not shown). In addition, in an attempt to bias in favor of selecting mouse OR pseudogenes, we searched for mouse OR sequences homologous to human pseudogenes. One member was chosen from three different OR pseudogene families: clones 1-72, 15-71, and 912-47 from chromosomes 1, 15, and 11, respectively (3). Each of these genes belongs to one of the three main groups of human OR sequences and has accumulated a number of mutations such as stop codons and indel frameshifts (3). The amino acid sequence identity between these three ranges from 31% to 41%.

High-density filters from the mouse OR sublibrary were then hybridized separately with the three human pseudogene probes at a high stringency; 14 clones were sequenced on both strands. These sequences showed 38% to 53% ASI to the human sequences used to select them, indicating that they are not the orthologs of the human pseudogenes. All have an uninterrupted ORF from TM2 to TM7 (not shown). Together, we sequenced 33 mouse OR sequences, none of which contained characteristic features of pseudogenes.

## Discussion

What is the basis for the differences in olfactory ability observed among mammals? Diverse reasons have been suggested, i.e., the size of the anatomical structures devoted to olfaction (e.g., olfactory epithelium, olfactory bulb, and cortical structures), the number of OR families/subfamilies, and the total number and diversity of expressed OR genes. The olfactory epithelial surface of macrosmatic animals such as dogs is larger than that in microsmatic humans (see ref. 4 and references therein). On the other hand, by using unique dog sequence probes that represent specific OR subfamilies that will not cross-hybridize with other subfamilies, comparative analyses have been performed by Southern blot analysis among a panel of mammals including dog and human. This study



indicates that the number of OR sequences per subfamily is similar in microsmatic and macrosmatic animals (4). Recently, we demonstrated that a high fraction (>70%) of the human OR genes have lost function during evolution and are represented as pseudogenes (3). We found that chromosomes 7, 16, and 17 contain a high fraction of potentially coding OR sequences, whereas other chromosomes, such as chromosome 3 (3, 20) or 11 (3), contained primarily pseudogenes. Other studies on chromosome 17 (14, 21) and on chromosome 11 (22) in which 75% of the OR sequences identified were pseudogenes support these observations. These findings led us to hypothesize that the number of functional OR genes could be correlated to the olfactory capability of a given animal. In an attempt to test this hypothesis, we sampled the OR gene repertoire of the main primate species, thought to be microsmatic, and of mouse, thought to be macrosmatic. Our analysis clearly indicates that (see Fig. 1 and Table 1), from New World monkeys to hominoids, there is an increase in the percentage of OR pseudogenes from  $\approx 0$  to  $\approx 70\%$ , with the highest pseudogene content observed in gorilla, chimpanzee, and human. Supporting this observation, during the course of this work, Sharon and colleagues (23) published a study about the evolution in primates of the OR genes orthologous to the human OR gene cluster located on chromosome 17p13.3 (14, 21). The authors conclude that a rapid decline ( $\approx 10$  million years ago, corresponding to the radiation of hominoids) of the functional OR repertoire occurred in mammals. In addition, we showed in a recent study that the pool of pseudogenes is still growing in human, which will probably evolve toward a minimal set of functional OR genes (13). It is therefore likely that there is a selective advantage for New World monkeys to retain a high proportion of functional OR genes, whereas this advantage seems to be reduced in Old World monkeys. The prosimian branch represented by two lemur species does not follow this rule and has accumulated a high fraction of OR pseudogenes (Table 1). This particular taxon is localized in Madagascar and is composed of diurnal (as EFU) and nocturnal (as ERU) animals, both of which are thought to have evolved from a common ancestral species. The two species showed no striking difference in the OR pseudogene fraction, suggesting that loss of functional OR genes preceded their divergence. Nevertheless, despite the number of OR families and subfamilies presented in this study (Fig. 2), the collection of sequences of the present work represents only a subset of OR genes, and it is still possible that the fraction of functional OR genes and OR pseudogenes could be different in other segments of the OR family in different species. However, although some studies indicate that mice and dogs have

**Fig. 2.** Comparison of the deduced protein OR sequences obtained from the different primate species characterized in this study. The dendrogram was established with the PILEUP program from the GCG Package. Percentage of ASI was determined by pairwise sequence comparisons with the GAP program and is indicated along the abscissa of the tree. Sequences from the literature are indicated by asterisks. Human OR sequences derived from the use of the OR5B-OR3B primers and representing the main OR families were selected from refs. 3 and 13. Dog (CfOLF1, its human counterpart HsOLF1, and CfOLF2) and chicken (COR4) sequences were selected from refs. 4 and 7, respectively. OR families (greater than 40% ASI) are indicated by pink circles, and subfamilies (greater than 60% ASI) are indicated by yellow squares. The main family was arbitrarily named family 1 and subdivided into two subfamily groups, 1-I and 1-II (ovals). Group 1-II comprises subfamilies A and B. Beside the sequence names, green dots indicate sequences derived from the use of the OR5B-OR3B consensus primers; red squares indicate sequences derived from the OR3.1-7.1 consensus primers; and blue rectangles indicate potentially functional genes (uninterrupted ORFs). In the case of HSA 912-93 (blue rectangle and double asterisk), this sequence contains only one nonsense point mutation in human but is potentially coding in other primates (ref. 13; see also Table 1).

Table 1. Fraction of pseudogenes in the OR gene repertoire of primate species and mouse

Family/species	No. sequences analyzed	Percentage ORF	Percentage pseudogenes	Average percentage pseudogenes by family
Hominoids				50
Human (HSA)	99	30	70	
Chimpanzee (PTR)	21	52	48	
Gorilla (GGO)	18	50	50	
Orangutan (PPY)	23	61	39	
Gibbon (HLA)	22	59	41	
Old World monkeys				27
Macaque (MSY)	20	65	35	
Baboon (PPA)	21	81	19	
New World monkeys				2
Marmoset (CJA)	19	100	0	
Squirrel monkey (SSC)	15	100	0	
Squirrel monkey (SBO)	15	93	7	
Prosimians				37
Lemur (EFU)	19	58	42	
Lemur (ERU)	16	69	31	
Rodents				0
Mouse (MMU)	33	100	0	
Fish				0
Zebrafish (DRE)	3	100	0	

increased olfactory abilities compared with humans (10, 24), accurate experiments to compare the olfactory ability between the different primates species remain to be conducted to support our hypothesis.

All OR sequences we derived from mouse are potentially coding. No pseudogenes were detected either by sequencing randomly selected OR sequences or by deliberately screening with human OR pseudogene probes. This result indicates that the OR pseudogene content is either zero or restricted to rare examples in mouse (25).

Taken together, this study led us to hypothesize that the reduction of the sense of smell could correlate with the fraction of functional OR genes in the genome. This phenomenon would probably result from the relaxation of the selective pressure exerted on the different species, i.e., as soon as the function becomes nonessential for the survival or the social behavior of a particular species, the genes responsible for that function tend to accumulate deleterious mutations. Actually, it is likely that the different hypotheses evoked to explain the loss of function would be not exclusive but that a parallel might exist between the reduction of the anatomical structures devoted to olfaction, the decrease in the number of functional OR genes, and the reduction of the sense of smell. This hypothesis is strongly supported by the fact that aquatic mammals such as dolphin, which has a reduced olfactory apparatus, have only OR pseudogenes (15). These animals live in water and do not need to smell volatile odorants. Therefore, a parallel degeneration of the olfactory organs and the OR gene repertoire has occurred probably because of the relaxation of the selective constraints. This sort of observation has also been made in the blind marsupial mole, in which degeneration of the eyes is accompanied by mutation of the interphotoreceptor retinoid binding protein gene, which is involved in the regeneration of rhodopsin in the visual cycle (26).

However, it is difficult to measure and compare the olfactory efficiency of different animal species. Various parameters such as the threshold of detection of odorants (sensitivity), the range of odors detectable, and the discriminatory power (acuity) are key parts of the olfactory ability. Thus, it is difficult to determine precisely which of these parameters are taken in account when comparing two species, and therefore the origin of the olfactory

deficiency of primates remains a controversial and difficult point to address. Furthermore, there are no data available in the literature comparing the olfactory ability between the different primate species studied in this work, and most of the few studies conducted to compare the performances of mouse or dog versus human concern either the threshold of detection of very few odorants (for example *n*-amyl acetate; ref. 27) or the observation that trained dogs are far better than humans in detecting hidden objects such as mines, drugs, or people buried after natural disasters (28, 29). Nonetheless, although the fraction of OR pseudogenes has been estimated in mouse, it remains to be determined in dog.

The chromosomal distribution of the OR gene repertoire arose through multiple duplication rounds (3, 5, 20, 30, 31) giving rise to paralogous regions. Even though the number of duplication events may be different among the mammals, overall it seems that the number of OR genes was established before the divergence of mammals (4). This observation explains why, as determined by Southern analysis, there is no striking difference in the number of OR genes of four different subfamilies between the sea lion, which has an underdeveloped olfactory apparatus, and other mammals (4). On the other hand, the Southern blot approach does not indicate the functionality of the OR sequences, and we predict that a large fraction of the sea lion OR genes could be pseudogenes as described for the dolphin (23). Similarly striking differences have been observed in the olfactory abilities of different breeds of dogs (12). Despite the variations in the size of the olfactory epithelium of the different breeds (ref. 12 and references therein), it would be interesting to determine the biological basis for the differences in performances between sight and scent hounds. One obvious possibility is loss of functional OR genes; however, given the recent origin of all modern dogs, this explanation seems unlikely. Other explanations could be changes in behavior, in expression brought about by the modification of a key master transcription factor, or in the unusual mechanism that allows only one OR gene allele or the other to be expressed exclusively in any one epithelium cell.

Finally, we hypothesize that the study of the evolution of the OR gene repertoire through the determination of the pseudo-

gene fraction could mirror the evolution of the olfactory sensory function in microsmatic and macrosmatic mammals.

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